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Prime solutions for cardiopulmonary bypass in neonates: Antioxidant capacity of prime based on albumin or fresh frozen plasma

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Objective: Oxidative damage and inflammation are believed to play an important role in postoperative complications after cardiopulmonary bypass. During bypass, a prime solution with a high antioxidant capacity may reduce the oxidative damage and inflammation. We investigated total antioxidant capacity and individual scavengers during the preparation of 2 different prime solutions.

Methods: The prime solutions were prepared with either pasteurized human albumin or fresh frozen plasma. The total antioxidant capacity was measured with the total radical antioxidant parameter assay and with the ferric-reducing ability of plasma assay. The individual scavengers vitamin C, sulfhydryl groups, uric acid, and total protein were measured before, during, and after the prime preparation. Malondialdehyde was measured as a parameter for lipid peroxidation.

Results: Neither prime solution showed a total radical antioxidant parameter value. The ferric-reducing ability of plasma value of prime solutions was lower than that of undiluted human albumin or fresh frozen plasma. Addition of mannitol did not increase the ferric-reducing ability of plasma value. Vitamin C was only found in the fresh frozen plasma prime. Both prime solutions contained sulfhydryl groups and uric acid in low concentrations. During ultrafiltration, low-molecular-weight antioxidants were lost into the ultrafiltrate.

Conclusions: We showed that prime solutions based on either albumin or fresh frozen plasma had very low antioxidant capacity and that ultrafiltration of the prime solution further lowers this capacity. A prime solution with a low antioxidant capacity may increase oxidative stress in neonates undergoing cardiopulmonary bypass.

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Oxidative damage and inflammation are believed to be important causes of morbidity related to cardiopulmonary bypass (CPB), which is high, especially in small infants.[1] During CPB, prooxidative substances, such as nonprotein-bound iron, are released while the plasma antioxidant capacity decreases, resulting in excess accumulation of reactive oxygen species.[2] This may be especially important in neonates who, compared with more mature patients, already have low plasma iron-binding capacity and poor antioxidant defenses, which decrease even further after CPB.[3,4] This may cause oxidative stress, direct tissue damage, and multiorgan failure. Moreover, oxidative stress may upregulate the inflammatory response and initiate a vicious oxidative circle.[1,5] Enhancing the antioxidant capacity of patients during CPB could limit the direct oxidative tissue

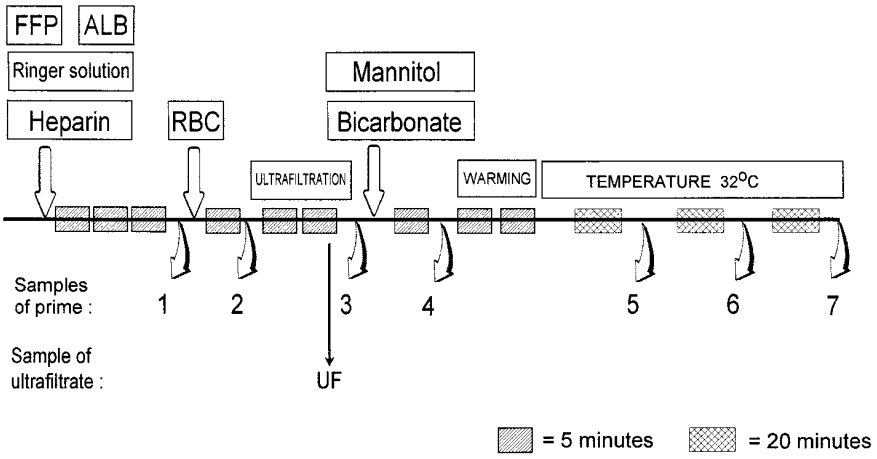


Figure 1. Composition, preparation, and sampling of the primes. *1*, Clear prime; *2*, RBC prime before ultrafiltration; *3*, RBC prime after ultrafiltration; *4*, RBC prime after mannitol and bicarbonate; *5*, *6*, and *7*, RBC prime after 20, 40, and 60 minutes, respectively, at a temperature of 32°C. Sample of ultrafiltrate was collected at the end of ultrafiltration.

damage and modulate the undesired inflammatory response. The prime solution of the CPB system can substantially affect the plasma antioxidant capacity because of the high ratio between prime and circulating volume of neonatal patients. As previously reported, even transfusion of a relatively small volume of fluid with a low antioxidant capacity decreases the ability of plasma of neonates to catabolize reactive oxygen species.[6] Thus, a large prime volume with a low antioxidant capacity may dramatically decrease the antioxidant capacity of neonates undergoing CPB. On the other hand, a supplementation of the prime solution with antioxidants could decrease the negative effect of the dilution and limit the oxidative stress during CPB.[7-9]

Albumin-based prime solution, which is routinely used in our institution, may have a lower antioxidant capacity than an alternative prime solution based on fresh frozen plasma (FFP). Ultrafiltration during prime preparation could change the antioxidant status of prime solution by means of removal of water-soluble antioxidants or prooxidative substances, such as nonprotein-bound iron. Therefore, we investigated the total antioxidant capacity, as well as that of selected individual antioxidants, during the preparation of these 2 different prime solutions.

Materials and Methods

This in vitro study was approved by the Scientific Committees of the Department of Pediatrics and the Department of Thoracic Surgery. Preserved packed red blood cells (RBCs; stored for <5 days) and FFP were delivered by our blood bank. RBCs were preserved and stored in saline, adenosine, glucose, and mannitol (SAGM) solution. FFP contains citrate, which is used as an anticoagulant during donor blood preparation. Informed consent of the donors was obtained. Twenty percent human albumin solution was obtained from CLB (Amsterdam, The Netherlands). This is a plasma-derived product prepared by means of ethanol fractionation and pasteurization (10 hours at 60°C). It contains mainly albumin (95%), but other proteins are also present, such as prealbumin and haptoglobin.

Prime Composition and Preparation

Prime composition and preparation are shown in Figure 1. Two different prime solutions on the basis of either albumin or FFP were prepared, each on 5 separate occasions. At room temperature, the cardiotomy reservoir of a Dideco Lilliput 901 CPB system (Dideco, Mirandola, Italy) was filled with 500 mL of Ringer's solution with 1500 IU of heparin and either 100 mL of 20% human albumin (ALB prime) or 100 mL of FFP (FFP prime). The oxygenator was filled, and after 15 minutes of circulation, 100 mL of packed RBCs were added to the clear ALB or FFP prime. After 5 minutes of circulation of this RBC prime, ultrafiltration was performed with a Minntech Hemocor HPH 400 (Minntech Corp, Minneapolis, Minn) to reduce the prime volume to 350 mL. Then 1.0 g of mannitol and 4.0 mL of 8.4% sodium bicarbonate were added. Five minutes later, the temperature of the prime was increased to 32°C for 60 minutes. (In a clinical situation, this decreases the temperature difference between prime and patient.) The flow of the prime and the air flow (fraction of inspired oxygen = 0.21) was 0.50 L/min. The flow through the ultrafilter was 0.20 L/min, with a constant pressure of 75 mm Hg.

Samples

Samples of undiluted albumin solution and FFP were collected. Samples (3 mL) of the prime at various stages of its preparation and one sample of ultrafiltrate were collected, protected from light, immediately cooled, and transported to the laboratory to be centrifuged (4°C for 5 minutes at 2000 rpm). For measurements of vitamin C, the samples were stabilized with metaphosphoric acid and deferoxamine mesylate (Desferal). The samples were frozen until analysis (–80°C under argon). Preliminary studies showed that values did not change during storage.[3]

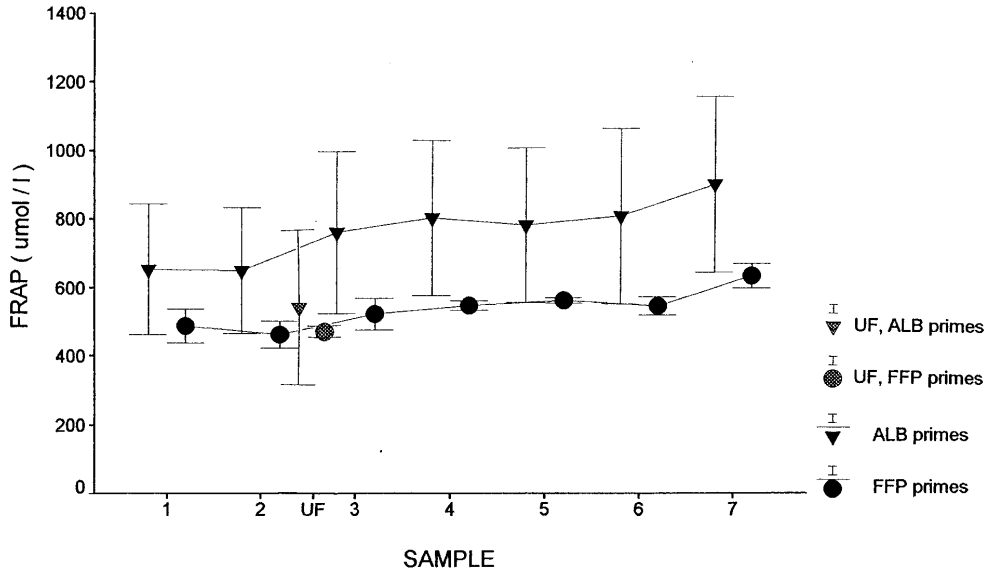


Figure 2. Measurement of FRAP assay (mean ± SD; circulation 6 excluded). Sampling is described in the legend for Figure 1. *UF,* Ultrafiltration.

Laboratory Measurements

The total antioxidant capacity was measured with 2 different methods.

The total radical antioxidant parameter (TRAP) assay measures the ability of the investigated sample to inhibit peroxidation of the target lipid (linoleic acid) induced by peroxyl radicals. This lipid peroxidation is measured by means of oxygen consumption with an oxygen electrode. Extent of the inhibition, if any, is quantified by using Trolox (water-soluble analog of vitamin E) as a calibrator.[6,10]

The ferric-reducing ability of plasma (FRAP) assay measures the capacity of the sample to reduce ferric ion (Fe^{3+}) to the ferrous form (Fe^{2+}). This reduction can be measured by means of spectrophotometry because ferrous ions bind to tripyridyltriazin to form a blue-colored complex. The results of the assay are quantitated by use of a solution containing ferrous ions in a known concentration as a calibrator.[11]

Individual Antioxidants

Vitamin C was measured with high-performance liquid chromatography, as previously described.[12-14] This method measures total ascorbic acid and its oxidized form, and reduced ascorbic acid is calculated by subtraction. Knowing the concentration of vitamin C and the exact (corrected for sampling) volume of prime, we calculated the total amount of vitamin C in the prime. Sulfhydryl groups were determined by spectrophotometry, as previously described.[15] Uric acid, total protein, and albumin were measured by means of an automatic analyzer (Hitachi 747; Roche Diagnostics GMBH, Mannheim, Germany).

Lipid Peroxidation Product

Malonyldialdehyde (MDA) was measured by means of high-performance liquid chromatography, as adapted and modified from the previous study.[16]

Statistics

All results are reported as means ± standard deviation (SD). Differences between oxidized/total ratio of vitamin C before and after FFP prime preparation were tested by using the *t* test for paired samples. Differences between means of the amount of vitamin C present in the prime in different samples were measured by 1-way analysis of variance. Differences between the amount of vitamin C present in FFP prime before and after ultrafiltration were tested by using a paired *t* test. Correlation between free hemoglobin/heme concentration and oxidized/total vitamin C ratio was tested with the Pearson method by the 2-tailed test of significance.

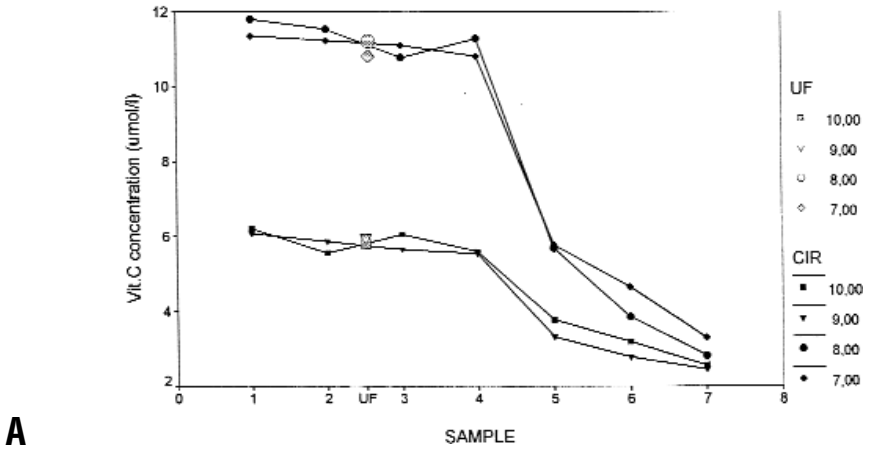
Results

The samples from one FFP prime preparation became hemolytic during centrifugation. After box-plot analysis, we excluded the results of these samples from FRAP and vitamin C statistic analysis.

Total Antioxidant Capacity

ALB prime. The TRAP assay detected no antioxidant capacity in either undiluted albumin solution or in the samples of the prime. The FRAP value in the undiluted albumin solution was 1813.7 μmol/L. In the clear prime, the FRAP value was 651.6 ± 190.8 μmol/L and increased during the procedure, reaching a value of 901.2 ± 255.9 μmol/L. The ultrafiltrate had a FRAP value of 542.5 ± 225.3 μmol/L (Figure 2).

FFP prime. The TRAP value of undiluted FFP was 752.75 ± 40.1 μmol/L; however, the assay detected no antioxidant capacity in the samples of the prime. The FRAP



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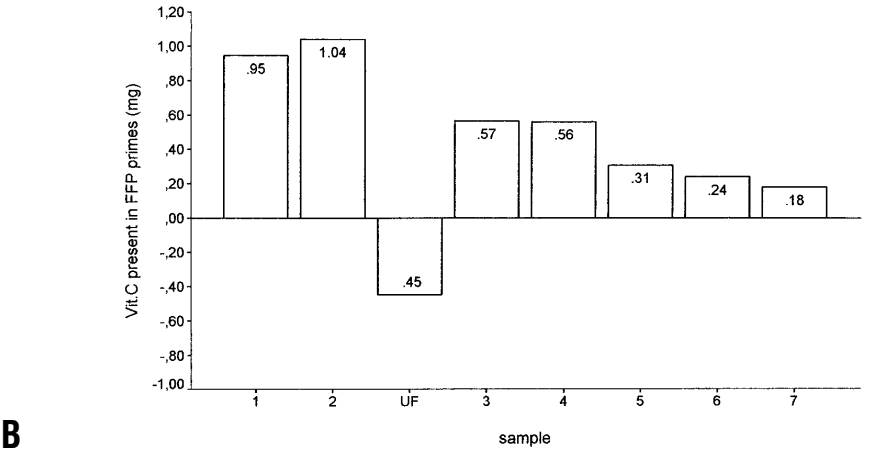

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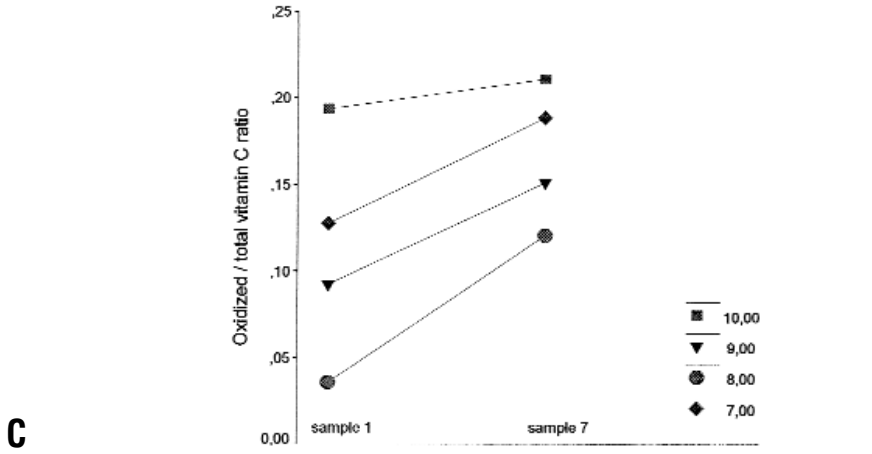

C

Figure 3. A, Individual vitamin C concentrations (in micromoles per liter) during the preparation of FFP prime (circulation 6 excluded). Sampling is described in the legend for Figure 1. Notice the large variability in concentrations between the different circulations *(CIR)* and especially the concentrations in the ultrafiltrates *(UF)*. B, The total load (mean) of vitamin C in milligrams during the preparation of the FFP primes (circulation 6 excluded). Sampling is described in the legend for Figure 1. Notice the loss of vitamin C during the whole preparation (paired *t* test: *P* = .017 for sample 1 and sample 7; before and after the preparation, respectively) and especially during ultrafiltration (paired *t* test: *P* = .019 for sample 2 and sample 3; before and after ultrafiltration, respectively). C, Measurements of oxidized/total ratio vitamin C (circulation 6 excluded). Sampling is described in the legend for Figure 1 (paired *t* test: *P* = .006 for sample 1 and sample 7).

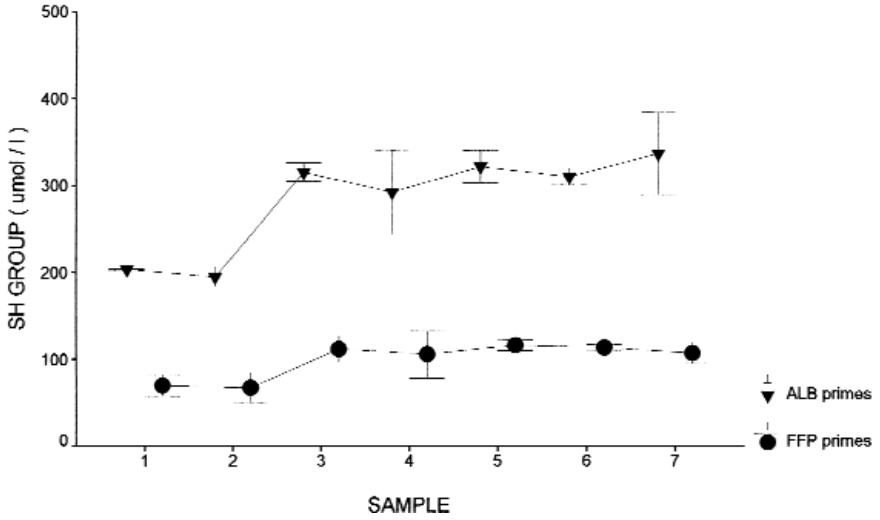

Figure 4. Measurements of sulfhydryl *(SH)* groups (mean ± SD). Sampling is described in the legend for Figure 1. No sulfhydryl groups were present in the ultrafiltrate.

value in the undiluted FFP was 2841.5 ± 703.2 μmol/L. In the clear prime the FRAP value was 486.9 ± 49.8 μmol/L and increased during the procedure, reaching a value of 635.3 ± 36.4 μmol/L. The ultrafiltrate had a FRAP value of 472.1 ± 17.4 μmol/L (Figure 2). Although there was a difference in FRAP values between the ALB and the FFP primes after the preparation, this difference was not significant (P = .08).

Vitamin C

ALB prime. No vitamin C was detected.

FFP prime. FFP prime contained vitamin C (Figure 3, *A-C*). Its mean concentration in the clear prime was 8.86 ± 3.15 μmol/L, ranging from 6.07 to 11.80 μmol/L and decreasing to 4.64 ± 1.27 μmol/L after 20 minutes of circulation of the prime at 32°C and to 2.78 ± 0.37 μmol/L after completion of the procedure (analysis of variance, P = .0035). In the ultrafiltrates, vitamin C was found in the same concentration as in the primes. Because of this, the total amount of vitamin C in the prime decreased during ultrafiltration from 1.04 ± 0.40 mg to 0.57 ± 0.20 mg. During the circulation of the prime at 32°C, the total amount of vitamin C decreased even further to 0.18 ± 0.03 mg after completion of the procedure. The oxidized/total vitamin C ratio increased from 0.11 ± 0.07 in the clear prime to 0.17 ± 0.04 after completion of the procedure. The ratio was positively correlated with free hemoglobin/heme concentrations in the primes (r = 0.88-0.83 and 0.83-0.69, respectively). Interestingly, in the hemolytic samples the ratio increased to 0.49 after the preparation and was strongly correlated with concentrations of heme (r = 0.89 and P = .003, data not shown).

Sulfhydryl Groups

Sulfyhdryl data are shown in Figure 4.

ALB prime. Clear prime contained sulfhydryl groups at a concentration of 203.4 ± 0.9 μmol/L. This concentration did not change after addition of RBCs but increased after ultrafiltration to 315.8 ± 10.9 μmol/L and remained stable until completion of the preparation. Ultrafiltrate contained no sulfhydryl group. Calculated sulfhydryl/total protein ratio was 4.74 ± 0.20 in clear prime and 5.53 ± 0.77 after completion of prime preparation.

FFP prime. Clear prime contained sulfhydryl groups at a concentration of 70.1 ± 12.2 μmol/L. This concentration did not change after adding RBCs but increased slightly after ultrafiltration to 111.8 ± 15.2 μmol/L and remained stable until completion of the preparation. The ultrafiltrate contained no sulfhydryl group. Calculated sulfhydryl/total protein ratio was 5.70 ± 1.19 in clear prime and 5.77 ± 0.64 after completion of prime preparation.

Uric Acid

ALB prime. Clear prime contained no uric acid. After adding RBCs, uric acid was detectable at a concentration of 0.01 ± less than 0.001 mmol/L (mean ± SD) and increased to 0.02 ± 0.01 mmol/L at the end of preparation. In ultrafiltrate, uric acid was found at a concentration of 0.01 ± 0.01 mmol/L.

FFP prime. Clear prime contained uric acid at a concentration of 0.04 ± less than 0.001 mmol/L and remained stable until the temperature increased and then increased to 0.06 ± 0.01 mmol/L after completion of prime preparation. In the ultrafiltrate, uric acid was found in the concentration of 0.04 ± less than 0.001 mmol/L.

TABLE 1. Plasma concentrations of the various plasma antioxidants compared with the concentrations in the prime solutions after the prime preparation procedure

	Term babies	Adults	ALB prime	FFP prime
Uric acid (μmol/L)	317 ± 66	285 ± 55	0.02 ± 0.01	0.06 ± 0.01
Vitamin C (μmol/L)	130 ± 55	62 ± 16	ND	2.78 ± 0.37
Sulfhydryl groups (μmol/L)	422 ± 80	496 ± 57	315.8 ± 10.9	111.8 ± 15.2

Data are presented as means ± standard deviation. Data for term babies and adults are adapted from reference 28. *FFP,* fresh frozen plasma; *ND*, not detectable.

Total Protein and Albumin

ALB prime. Clear prime contained 42 ± 1.8 g/L protein (consisting of albumin, although we did not exclude traces of other proteins). Addition of RBCs slightly decreased the concentration of protein, and ultrafiltration increased it to 62 ± 2 g/L. After that, the protein content remained stable.

FFP prime. Clear prime contained 12 ± 0.9 g/L protein. Adding RBCs slightly decreased the concentration of protein, and ultrafiltration increased it to 18.4 ± 0.9 g/L. After that, the protein content remained stable. Protein in all FFP samples consisted of 55% to 60% albumin.

Malonyldialdehyde

ALB prime. Clear prime contained MDA at a concentration of 0.43 ± 0.05 μmol/L. This concentration increased slightly after adding RBCs (0.47 ± 0.1 μmol/L) and much more after ultrafiltration to 0.66 ± 0.08 μmol/L and slightly decreased during circulation at 32°C, reaching 0.62 ± 0.1 μmol/L after completion of the preparation. The ultrafiltrate contained MDA at a concentration of 0.13 ± 0.02 μmol/L.

FFP prime. Clear prime contained MDA at a concentration of 0.19 ± 0.07 μmol/L. This concentration increased slightly after adding RBCs to 0.28 ± 0.02 μmol/L and after ultrafiltration to 0.31 ± 0.05 μmol/L and remained stable until completion of the preparation. Ultrafiltrate contained MDA at a concentration of 0.12 ± 0.04 μmol/L.

Discussion

CPB was reported to completely deplete the total antioxidant capacity in neonates.[4] However, a possible effect of the composition of prime solution and its dilution effect on plasma of patients was not discussed. In our experience, even transfusion of a relatively small volume of pasteurized plasma protein solution, a plasma-derived product with no TRAP value, can decrease the antioxidant capacity of plasma of neonates.[6] The ratio between the prime volume and the circulating volume of neonates is much higher than during transfusion. As a result, prime solution with a low antioxidant capacity could substantially decrease the ability of these patients to metabolize reactive oxygen species. We therefore studied the antioxidant capacity of 2 different prime solutions.

We showed that both ALB and FFP prime solutions have a low total antioxidant capacity. TRAP assay revealed no antioxidant capacity in ALB prime, reflecting the fact that human albumin solution has no TRAP value. This is probably because of aggressive processing of donor plasma causing damage or loss of antioxidants during manufacturing of this plasma-derived product. The TRAP value of FFP prime was also undetectable, probably as a result of dilution of donor plasma, which had a normal TRAP value. The FRAP value of human albumin solution was originally much lower than that of FFP. However, the FRAP value of the clear ALB prime was, surprisingly, slightly higher than that of FFP prime. This suggests a different effect of dilution of human albumin solution and FFP or an interaction with heparin. Heparin is believed to have an antioxidant activity; however, its exact mechanism is not clear.[17] Moreover, measurement of total antioxidant capacity can be influenced by heparinization of samples, giving higher apparent results.[18] In FFP prime this effect of heparin can be lower because of its binding to antithrombin III in donor plasma. In ALB prime, unbound heparin can possibly increase the results of the FRAP assay. Relatively high FRAP values of ultrafiltrates of both primes suggest that this assay mainly measures the effect of low-molecular-weight antioxidants. Interestingly, the hemolytic samples had very high FRAP values. Mannitol, which is widely used as an important hydroxyl radical scavenger, did not improve the total antioxidant capacity of the prime solutions.[19,20]

For further insight into the antioxidant properties of the prime solutions, we also investigated a few selected antioxidants during the prime preparation.

Vitamin C, an important secondary antioxidant, was not detected in ALB prime, probably because of its loss during the production process of albumin solution. There was a large variability in vitamin C concentrations between the different preparations of the FFP prime, probably because of donor variability. These differences became small after the prime preparation. The concentrations remained stable after adding RBCs, bicarbonate, and mannitol but decreased sharply during circulation at 32°C. RBCs rapidly take up oxidized vitamin C and slowly release the reduced form of vitamin C.[21] This active uptake of vitamin C may explain the decrease of

concentration of total vitamin C. Concomitantly, with a decreasing concentration of vitamin C, its oxidized/total ratio increased, suggesting a rise in oxidation or less effective recycling of oxidized vitamin C. Oxidation of vitamin C can be explained by interaction with pro-oxidative free hemoglobin/heme present in the prime. Glutathione plays a crucial role in the recycling of oxidized vitamin C.[22] The ability of RBCs to maintain glutathione is diminished during the prime preparation (unpublished data). This can result in decreased recycling of vitamin C and increasing of its oxidized/total ratio in the prime solution. Ultrafiltration did not change the concentration of vitamin C; however, its total amount in the prime decreased because of loss into the ultrafiltrate.

Uric acid is the most important antioxidant and contributor to the TRAP value of human plasma.[6,10] Both primes contained very low concentrations of uric acid. However, it was much higher in FFP prime than in ALB prime. Uric acid in FFP prime originated from donor plasma, and its low concentration is an effect of its dilution. Clear ALB prime contained no uric acid, and RBCs added to the prime contributed to its minimal concentration thereafter. The concentration of uric acid in ultrafiltrate was the same as in the primes, indicating that this small molecule was (similarly to vitamin C) freely filtered out during ultrafiltration.

Proteins used to maintain the colloid osmotic pressure during CPB (especially albumin) can contribute to the antioxidant capacity of the primes. Albumin binds pro-oxidative heme and transition metals, whereas its sulfhydryl and, as recently reported, hydroxyl groups can act secondarily (eg, scavengers and antioxidants).[23,24] Moreover, human albumin solution also contains some haptoglobin, which binds potentially pro-oxidative hemoglobin.[25] ALB prime has higher protein (albumin) contents than FFP prime, which is a logical consequence of their composition. High albumin concentration in ALB prime could improve its antioxidant capacity.[23] Plasma sulfhydryl groups are mainly present in the cysteine components of proteins and in low concentrations in glutathione. However, the sulfhydryl/protein ratio was lower in ALB prime than in FFP prime, and we found free heme and nonprotein-bound iron in ALB prime (unpublished data). This can be explained by oxidation of sulfhydryl groups as a result of plasma processing during the production of albumin solution.

MDA is a product of lipid peroxidation but can also attack proteins and DNA.[26] It was present in both clear primes in very low concentrations because of the effect of dilution of donor plasma and albumin solution. Concentrations of MDA in ultrafiltrate were much lower than in the primes, indicating that MDA, despite its small molecular size, was not effectively ultrafiltered. MDA can bind to proteins, which probably prevents its ultrafiltration.

We saw hemolysis during centrifugation in the samples from FFP prime. Interestingly, when the same RBCs were used for ALB prime, no hemolysis was seen. It is possible that these particular RBCs were more susceptible for factors that could be stronger in FFP prime than in ALB prime. No TRAP value, lower FRAP value, the presence of vitamin C, a lower concentration of protein/albumin, and sulfhydryl groups in the FFP prime can be explanations for the hemolysis.

We used ultrafiltration during the preparation of the primes in an attempt to decrease the metabolic load from preserved RBCs and to reduce the prime volume.[27] In this study we showed that ultrafiltration also removes important low-molecular-weight antioxidants, such as vitamin C and uric acid. Their concentrations were not changed; however, the total amount of these antioxidants in the prime decreases. This implies that ultrafiltration during and after CPB may reduce the amount of important low-molecular-weight antioxidants in the circulation of the patient. However, we showed that despite the loss of these low-molecular-weight antioxidants, the total antioxidant capacity of the prime is not decreased after ultrafiltration.

In conclusion, we showed that both ALB prime and FFP prime had no TRAP value. FRAP values were much lower than the values measured in the undiluted human albumin solution or in FFP prime. Mannitol did not improve the antioxidant capacity of the primes. During ultrafiltration, low-molecular-weight antioxidants were lost into the ultrafiltrate. Ultrafiltration was not able to decrease the concentration of MDA in the primes. Composition and antioxidant capacity of prime may substantially affect antioxidant capacity of neonates undergoing CPB. To emphasize the marked dilutional changes in antioxidant concentration that could occur, we compare the normal plasma antioxidant concentrations in terms of babies and adults with the concentrations found in prepared primes (Table 1).[28] If ultrafiltration is used during the prime preparation, attention has to be paid to supplementation of antioxidants to the prime after the use of ultrafiltration.

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